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Preliminary note on a differential staining of the cytoplasm of Characeae

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The observations here recorded were made principally on two species of *Nitella*, but they are believed to apply generally for plants of this family. Perhaps the means of differential staining of living cell contents will find useful application in the study of other plants, but thus far my attention has been confined to the charas. Although it is my intention to continue observations in this direction, this should not deter others from work on the same lines. The field opened for investigation by this means is large and of much interest.

It has long been known that the peripheral layer of cytoplasm in a developed internodal cell of *Chara* is relatively dense and viscous, while that within is notably thinner. By the use of neutral red the cell contents of a *Chara* plant may be differentially stained while the plant is living. The color is readily taken up from a dilute solution. An elongated internodal cell thus stained shows the cyclosis more clearly and beautifully than in the natural condition. Observing such a stained cell there is seen a large central cylinder of a pronounced cardinal red or wine color, bordered on either side with a narrow line of green.

Within the colored cylinder, or vacuole, are numerous suspended granules, vesicles and spherical elements of various kinds, the nature of which is, for the most part, undetermined. Some of these are deeply colored and can be more advantageously studied than in the natural state. Indeed, they take the stain before the vacuole shows a general coloration. Probably these various structures have never before been so clearly seen and under such favorable conditions for study.

The borders appear green because the light passes through the marginal chloroplasts regularly arranged next to the cellulose wall. But through this green sheen may be clearly seen the outer layer of denser cytoplasm, carrying small, uncolored granules and some spherical plasmic bodies in suspension, in active cyclosis, closely following the cell wall. This outer layer, which, for convenience, may be here specifically designated as protoplasm, is quite uncolored. Thus we have, if we imagine a cross section, the cellulose wall lined with the chloroplasts in a thin, reticulated stroma, then the thin layer of uncolored, moving protoplasm, within which is the strongly colored, wide cylinder.

The line of demarcation between the colored cylinder and the uncolored protoplasm is as sharply defined as a thin cell wall. Clearly it is the so-called vacuole wall. This line is wavy and constantly changing as it yields to the irregularities in the thickness of the protoplasm stream. The stained contents of the vacuole participate, however, in the movement of the protoplasm without, following precisely the same course. Moreover, deep within the vacuole it may be observed that the neutral or indifferent line, which marks the separation of the currents moving in opposite directions, indicates also an invisible barrier within the vacuole, which cannot be easily crossed by stray elements from the circulating stream.

A sharply defined differentiation of the cell contents has thus been effected. The significance of it is not yet clear. It is not possible at present to state precisely what particular parts or elements in the cell sap have taken the stain, or whether any color is held in solution. By far the greater number of the corpuscles or spherical elements so evident in the circulating stream of charas, are suspended in the cell sap, not in the protoplasm. The Schleimbläschen of Naegeli, later named Wimperkörperchen and mentioned by Allen as "ciliated" granules of protoplasm, are often deeply stained, but apparently not always. The name is misleading since there is no evidence of the presence of motile cilia on these bodies.

We have some admirable hypotheses concerning osmotic action in the plant cell. There are membranes permeable and selectively permeable, solutes obedient to the rules established for their guidance, whereby the whole train of physical phenomena becomes clear. It now becomes desirable to know how the coloring matter makes its way into the vacuole of the *Nitella* cell. The solution passes directly through the outer membrane and the rotating protoplasm, without coloring or affecting them in any way, so far as can be observed. It also passes through the vacuole wall. Apparently some ingenuity will be required to explain the observed phenomenon by osmotic action. One is inclined to regard it as indicating a general permeability of the tissues to the colored solution, which happens to be one not injurious to life processes. The color is strongly held in the vacuole and in uncolored water cyclosis continues for many days.

Perhaps it is true, as some have supposed, that living protoplasm will not take up coloring matter. We are told that when the nucleus stains the cell is dead. We have seen, however, that protoplasm permits passage of the coloring matter used in these experiments. Should we, then, conclude, that the contents of the vacuole which stain so deeply are therefore dead matter? Such a view is not sustained by the observed active circulation of the colored cell sap along the definite lines established.

A PECULIAR PLASMIC STRUCTURE IN THE NITELLA CELL

Among the many corpuscular elements of the cytoplasm, of which we have almost no understanding, some are of such remarkable character as to justify particular mention, although they cannot yet be satisfactorily described. They are curious spherical masses of granular matter, of extreme plasticity, greatly varying in size up to 0.1 mm. in diameter. The granules are in a state of constant agitation, as though the mass were seething with life. Within these spheres may be seen from one to twenty or more concave, saucer-shaped disks, 5–15 μ in diameter, with contours more or less irregular, in rapid rotation, like so many wheels. They move freely about in the sphere, constantly changing the plane of rotation, sometimes on edge, sometimes lying flat but always twisting about and changing position. The nature of the movement is suggestive of cilia, but thus far none have been detected.

These have seemed to me as perhaps connected with the development of chloroplasts, many of which are found freely circulating in the cytoplasm. Although this suggestion rests upon a very weak foundation, I find it difficult to resist the impression from observations up to this time.

These structures were first observed by me on June 18, 1918, in a variety of *Nitella opaca* Ag., found at Ithaca, New York, although not recorded in the local flora. Since then I have repeatedly observed them in another species of *Nitella*, not yet seen in fruit, and also in *Chara coronata*, var. *Schweinitzii*. From this it may be inferred the structures are common to this family of plants.

The only mention I have found in the literature of anything resembling these structures is in an admirable paper by Goeppert and Cohn,* where they are rather imperfectly described, not quite as I have seen them. Doubtless the description relates to the same structures. The authors associated them with the formation of starch.

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SUPPLEMENTARY NOTE

The long, hyaline rhizoids of *Nitella* afford a more favorable means of demonstrating the selective coloring. The vacuole of a rhizoid cell becomes deeply colored, while the thin, uncolored outer stream is in active rotation.

The remarkable fact, already mentioned, that only living cells become colored, is more clearly manifest in the small and transparent rhizoid cell. In any mass of rhizoids by far the greater number will be dead cells. These remain colorless in the dye, while the living cells begin to color immediately. So long as cyclosis continues in a cell, that cell will take the color. If the cell is dying and the cyclosis is weakening it will not stain so deeply as in active life; but whenever a trace of color is seen cyclosis can be detected. When cyclosis has ceased no coloration whatever is visible. When a stained cell dies the color soon disappears from the vacuole, doubtless by diffusion in the outer water.

It would seem as though we had in this a test for living matter! But what kind of living matter has the vacuole and how does it differ from the denser, outer protoplasm? Or, have we instead, a test for vitality in a cell? Speculation as to the significance of these facts is at present unprofitable. Osmotic action affords no explanation of a concentration of a solute by passing through a membrane; yet, clearly, the dye is taken from the dilute, outer solution and concentrated within the walls.

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* Ueber die Rotation des Zellinhaltes in *Nitella flexilis*. Bot. Zeit. 7: 665–673, 681–691, 697–705, 713–719. *pl. 10.* 1849.

With the printer's proof at hand I would like to add that the vacuole contents may be strongly colored by reason of an acid reaction. Ordinary water, or distilled water, usually is slightly alkaline, slowly changing neutral red to yellow or orange. When *Nitella* is grown in water slightly acidified with acetic acid, the vacuole becomes much more deeply colored than the culture solution.